Elite Marine Ballast Water Treatment System Corp.

Seascape®-BWMS Land-based Testing Sampling Testing Analysis Report

Testing Organization: The First Institute of Oceanography, State Oceanic Administration

Witness Institutions: China Classification Society, Nanjing Office

Development Organization: Elite Marine Equipment & Engineering Inc.

Testing Site: COSCO(Dalian) Shipyard

2012-8

Contents

1. INTRUCTION

Ships carry about 50-100 hundred million tons ballast water every year in the world (Endresen et al. 2004). There are particle sediments and all kinds of organisms including roe, fish larvae, bigger zooplankter (Williams et al. 1988; Carlton & Geller 1993), large alga and phytoplankton (Hallegraeff et al. 1997;Hamer et al. 2000;), also bacterium and virus etc. (Gollash et al. 1998). Under normal circumstance, these organisms live in port which ships set sail and around ecological system but not the destination port ecological system. In hundreds of the examples, due to these invasive alien species, a major harizonaards occurs to human health and environment. Not only destroy the balance of ecology but also suffer heavy losses in economy (Hoagland et al. 2002). Such as decreasing of commercial fishes and shellfish, causing cholera etc.. If don't take actions to deal with this problem, many reasons will cause invasive alien species increase in index. In the next decades, ships will become bigger and faster. Because more and more ships will across ocean, and it is a great opportunity for these species to mass propagates. Therefore, invasive alien species is considered as one of the main threatens in the world. In order to reduce this damage, International Maritime Organization (IMO) passed the ballast convention, 2004 (Anonymous 2005). The Convention requires all

the ships (>50,000 tons) should install appropriate ballast water management system (BWMS). In recent years, it has been reported and tested many BWMSs whose final aim is to reduce the organisms in the ballast water (Haskoning 2001;Rigby & Taylor 2001). There are more than twenty BWMSs have received the approval from IMO. The BWMS developed by Hyundai Group was installed on a VLCC successfully first time in 2011 (http://twitter.com/yonhapcn). In our country BWMS is still in testing period. And it will be a great effort to protect marine environment and prevent invasive alien species from propagating.

Now Elite Marine Equipment & Engineering Inc. authorizes us to examine their ballast water management system Seascape®-BWMS land-based testing samples.

2 LAND-BASED TESTING

2.1 Testing site

Land-based testing conducted in floating dock of COSCO (Dalian) Shipyard March, 2012. This floating dock meets the requirement of G8 for land-based testing. And Fig.2-1 is the photo of floating dock for testing. Detail information refers to Table 2-1.

Address: NO.80 Zhongyuan Road, Dalian city of Liaoning Province.

Fig. 2-1 Floating dock for testing

2.2 Testing tank

Twenty-six tanks of floating dock are used as testing raw water tank, treated tank and control tank. The tank arrangement is as following table 2-1.

Table 2-1 Tank arrangement for testing

Cycle	Raw water tank	Tank capacitym^3	Treated tank	Tank capacity m ^{3}	Control tank	Tank capacity m ^{3}
H1	NO.2W.B.T.(P)	4259.7	NO.2W.B.T.(C.FWD.)	2244.1	NO.2W.B.T.(C.AFT.)	2244.1
H2	NO.2W.B.T.(P)	4259.7	NO.3W.B.T.(C.FWD.)	1795.3	NO.3W.B.T.(C.AFT.)	1795.3
H3	NO.2W.B.T.(P)	4259.7	NO.4W.B.T.(C.FWD.)	1795.3	NO.4W.B.T.(C.AFT.)	1795.3
H4	NO.2W.B.T.(P)	4259.7	NO.5W.B.T.(C.FWD.)	1795.3	NO.5W.B.T.(C.AFT.)	1795.3
H5	NO.2W.B.T.(P)	4259.7	NO.6W.B.T.(C.FWD.)	1840.2	NO.6W.B.T.(C.AFT.)	1840.2
L1	NO.2W.B.T.(S)	4259.7	NO.8W.B.T.(C.FWD.)	1795.3	NO.8W.B.T.(C.AFT.)	1795.3
L2	NO.2W.B.T.(S)	4259.7	NO.7W.B.T.(C.FWD.)	2244.1	NO.1W.B.T.(C.AFT.)	1795.3
L3	NO.2W.B.T.(S)	4259.7	NO.3 W.B.T.(P)	3727.0	NO.3W.B.T.(S)	3727.0
L4	NO.2W.B.T.(S)	4259.7	NO.5W.B.T.(P)	3328.0	NO.5W.B.T.(S)	3328.0
L5	NO.2W.B.T.(S)	4259.7	NO.4W.B.T.(P)	3328.0	NO.4W.B.T.(S)	3328.0

During the testing progress, surveyor checked the hatch cover is covered and sealed with lead after the tank is cleaned. And the lead

sealing is opened after testing. We equipped every tank with nameplate to indicate the tank's use and number.

Table 2-2 Land-based testing basic information

Manufacture	Elite Marine Equipment & Engineering Inc.
Testing location	COSCO (Dalian) Shipyard (38°56′N，121°39′E)
Ballast tank capacity	about 1795.3-4259.7 m^3 each
Ballast tank quantity	26
Sampling principle	Isokinetic sampling
Inner structure	Common structure, including drain hole
Design, structure and surface coating	As per standard industrial practice

2.3 Sampling Point and Time

Three sampling points are set in the system (shown in the following diagrams). There are three sampling points in the system. The following are the details:

Sampling point 1: Testing environmental parameters and organisms of raw ballast water flowing into treating system (which can also be described as inflow water immediately before the treating equipment in accordance with G8 guidelines).

Sampling point 2:Testing environmental parameters and organisms of ballast water treated by the system (which can also be described as treated water immediately after the treating equipment in accordance with G8 guidelines).

Sampling point 3: Testing environmental parameters and organisms of ballast water flowing out of control tank after storing for 5 days and

treated water deballasted after stored for 5 days (which can also be described as treated ballast water that are stored in ballast tank for 5 days and upon discharge in accordance with G8 guidelines), respectively.

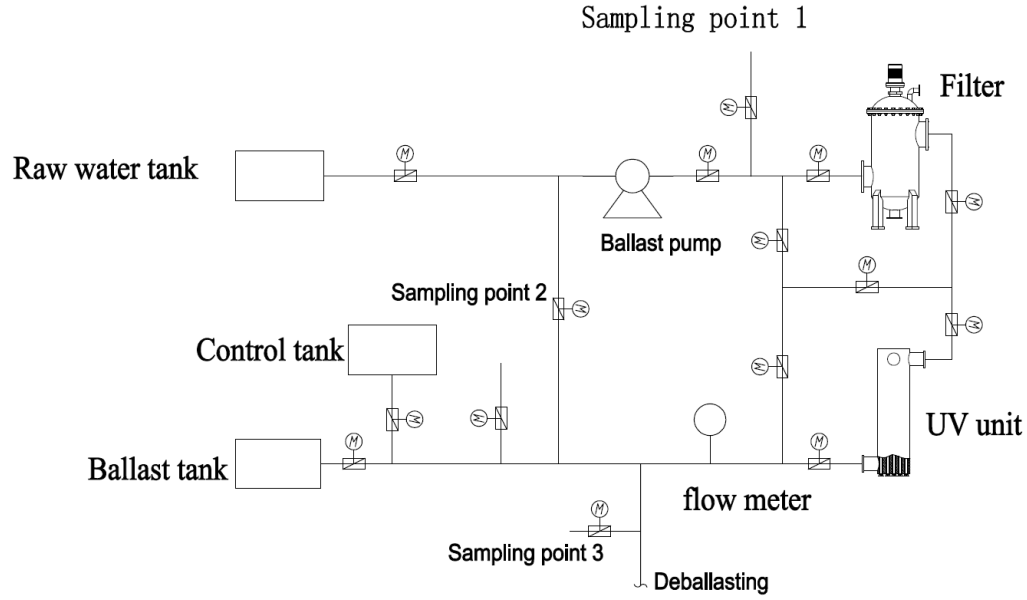


Fig. 2-2 Sampling point position

2.3.1 Sampling Principal

During sampling the characters and the velocity of the water won't be changed, which means the water samples are the same velocity with the sampling points in isokinetic condition and flow as they were. The sampler is designed in an individual bypass flow from the main flow. Therefore the water won't deviate or assemble when reaching the entrance of the sampler.

The formula can be shown as:

$$D_{iso} = D_m\sqrt{Q_{iso}/Q_m}$$

D_{iso} —— the diameter of the sample connection entrance;

D_m —— the diameter of the main flow in discharging pipelines;

Q_{iso} —— the volume of the sampling pipelines;

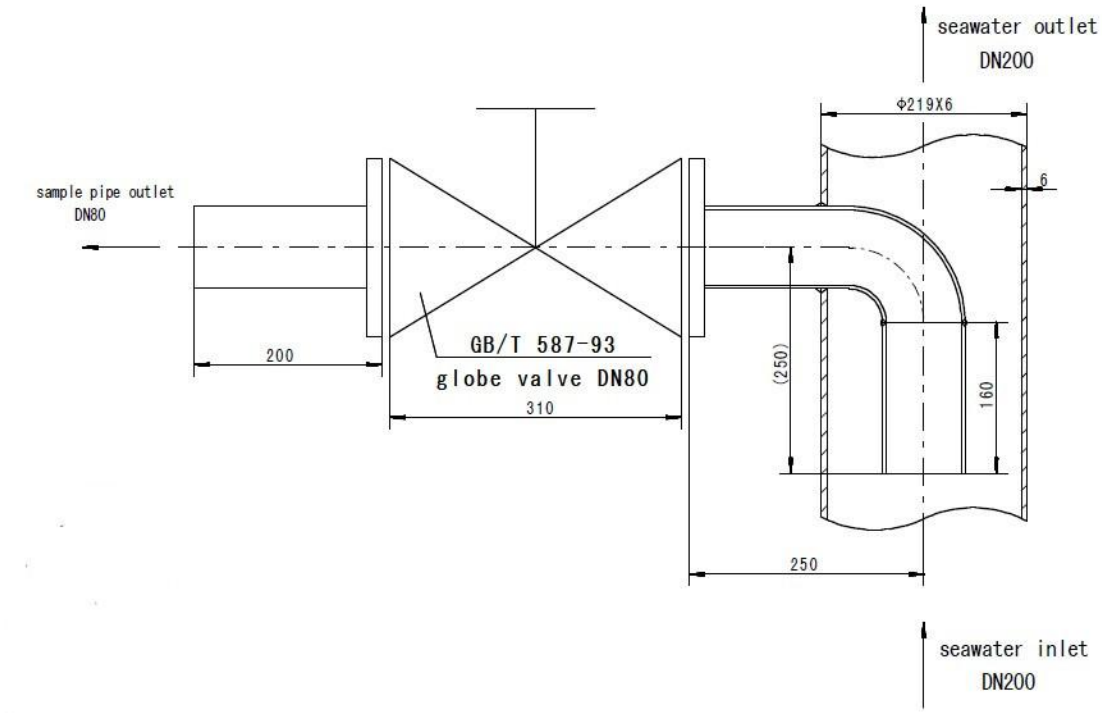
Q_m —— the volume of the discharging pipelines.

In this system, the Q_m is 250m^3/h, the D_m is 200 mm. During the land-based testing,

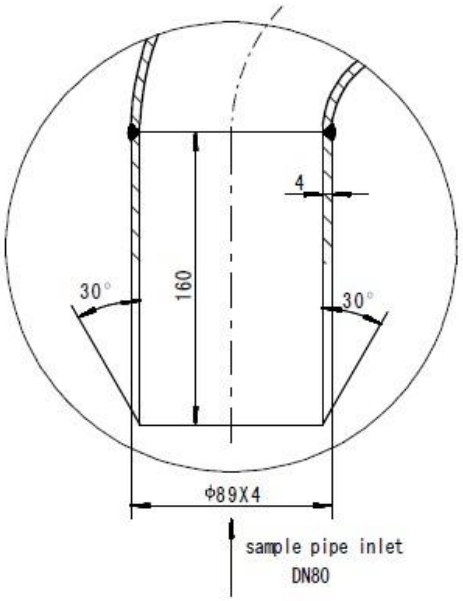
At sampling point 1, the sampling volume is 0.05 m^3 and time is 5 min, so the Q_{iso} is 0.6 m^3/h. From the above, the D_{iso} is 9.8 mm. The sample port diameter is designed to be 1.5 times the isokinetic diameter. i.e. 14.7 mm which is taken by 15 mm. The pipline's thickness is 4 mm.

At sampling point 2 and 3, the sampling volume is 1.5 m^3 respectively and time is 5 min, so the Q_{iso} is 18 m^3/h. From the above, the D_{iso} is 53.6 mm. The sample port diameter is designed to be 1.5 times the isokinetic diameter. i.e. 80.5 mm which is taken by 80 mm. The pipline's thickness is 4 mm.

The following is details of sampling pipelines at point 2 and 3:



(a) sampling point diagram



(b)details for sampling point

Fig.2-3 Sampling point details

2.4 Sampling Volume

About 0.05 m^3 raw water sample will be taken respectively at the beginning, middle and ending of the treatment at sampling point 1; about

1.5 m^3 water samples will be taken respectively at the beginning, middle and ending of the treatment at sampling point 2 and 3; all the water samples will be separated and tested according to the following requirements:

Table 2-3 Details for each sampling point

	Sampling time	Sampling volume	Sampling purpose
Sampling point 1 (raw water sample)	Beginning	Organisms for minimum dimension greater than 50 μm (20 L)	Testing organisms for minimum dimension greater than 50 μm
		Organisms for minimum dimension between 10 and 50 μm (1 L)	Testing organisms for minimum dimension between 10 and 50 μm
		Bacteria (500 mL)	Testing bacteria
		Environmental parameters (10 L)	Testing environmental parameters
	Middle	Organisms for minimum dimension greater than 50 μm (20 L)	Testing organisms for minimum dimension greater than 50 μm
		Organisms for minimum dimension between 10 and 50 μm (1 L)	Testing organisms for minimum dimension between 10 and 50 μm
		Bacteria (500 mL)	Testing bacteria
		Environmental parameters (10 L)	Testing environmental parameters
	End	Organisms for minimum dimension greater than 50 μm (20 L)	Testing organisms for minimum dimension greater than 50 μm
		Organisms for minimum dimension between 10 and 50 μm (1 L)	Testing organisms for minimum dimension between 10 and 50 μm
		Bacteria (500 mL)	Testing bacteria
		Environmental parameters (10 L)	Testing environmental parameters
Sampling point 2 (treated water sample)	Beginning	Organisms for minimum dimension greater than 50 μm (1 m^3)	Testing organisms for minimum dimension greater than 50 μm
		Organisms for minimum dimension between 10 and 50 μm (10 L)	Testing organisms for minimum dimension between 10 and 50 μm
		Bacteria (500 mL)	Testing bacteria
		Environmental parameters (10 L)	Testing environmental parameters
	Middle	Organisms for minimum dimension greater than 50 μm (1 m^3)	Testing organisms for minimum dimension greater than 50 μm
		Organisms for minimum dimension between 10 and 50 μm (10 L)	Testing organisms for minimum dimension between 10 and 50 μm
		Bacteria (500 mL)	Testing bacteria
		Environmental parameters (10 L)	Testing environmental parameters
	End	Organisms for minimum dimension	Testing organisms for minimum

		greater than 50 μm (1 m^3)	dimension greater than 50 μm
		Organisms for minimum dimension between 10 and 50 μm (10 L)	Testing organisms for minimum dimension between 10 and 50 μm
		Bacteria (500 mL)	Testing bacteria
		Environmental parameters (10 L)	Testing environmental parameters
Sampling point 3 (stored water deballasting sample)	Beginning	Organisms for minimum dimension greater than 50 μm (1 m^3)	Testing organisms for minimum dimension greater than 50 μm
		Organisms for minimum dimension between 10 and 50 μm (10 L)	Testing organisms for minimum dimension between 10 and 50 μm
		Bacteria (500 mL)	Testing bacteria
		Environmental parameters (10 L)	Testing environmental parameters
	Middle	Organisms for minimum dimension greater than 50 μm (1 m^3)	Testing organisms for minimum dimension greater than 50 μm
		Organisms for minimum dimension between 10 and 50 μm (10 L)	Testing organisms for minimum dimension between 10 and 50 μm
		Bacteria (500 mL)	Testing bacteria
		Environmental parameters (10 L)	Testing environmental parameters
	End	Organisms for minimum dimension greater than 50 μm (1 m^3)	Testing organisms for minimum dimension greater than 50 μm
		Organisms for minimum dimension between 10 and 50 μm (10 L)	Testing organisms for minimum dimension between 10 and 50 μm
		Bacteria (500 mL)	Testing bacteria
		Environmental parameters (10 L)	Testing environmental parameters
Sampling point 3 (control sample)	Beginning	Organisms for minimum dimension greater than 50 μm (1 m^3)	Testing organisms for minimum dimension greater than 50 μm
		Organisms for minimum dimension between 10 and 50 μm (10 L)	Testing organisms for minimum dimension between 10 and 50 μm
		Bacteria (500 mL)	Testing bacteria
		Environmental parameters (10 L)	Testing environmental parameters
	Middle	Organisms for minimum dimension greater than 50 μm (1 m^3)	Testing organisms for minimum dimension greater than 50 μm
		Organisms for minimum dimension between 10 and 50 μm (10 L)	Testing organisms for minimum dimension between 10 and 50 μm
		Bacteria (500 mL)	Testing bacteria
		Environmental parameters (10 L)	Testing environmental parameters
	End	Organisms for minimum dimension greater than 50 μm (1 m^3)	Testing organisms for minimum dimension greater than 50 μm
		Organisms for minimum dimension between 10 and 50 μm (10 L)	Testing organisms for minimum dimension between 10 and 50 μm
		Bacteria (500 mL)	Testing bacteria
		Environmental parameters (10 L)	Testing environmental parameters

2.3.3 Contingency Plan for Sampling

1. Power or device stoppage

When this happens, manuall y start the equipment after power restoration and equipment troubleshooting; discharge the raw water overboard directly without treatment. Re-run the equipment under normal process after ten minutes and then continue to experiment.

2. Sampling equipment contamination

The test sites are equipped with standby sampling equipment during the test (such as the sampling barrel, etc.). When this happens, please replace the sampling equipment soonest to ensure test validity.

3. External factor interference

When external factors interference leads to invalid test during process, please eliminate interference promptly and then re-run the equipment.

4. Placement

During the test, 1-2 reserve personnel are available for each post, when the staff cannot be on duty properly, a replacement appointment will be called to ensure that the testing is carried out strictly and effectively.

2.4 Testing Plan

2.4.1 Pilot run

After Seascape-BWMS installation, the whole system would run 24 hours with local water on site to test its reliability (including pump, pipes and valves etc.). Before land-based testing, a normal cycling should be done to ensure the human resource is enough and everyone knows their responsibility. Therefore, all the preparation is ready for regular land-based testing.

2.4.2 Testing and Assessment of the Raw Water

1. The ballast water quality will be tested at first to determine the quantity of organisms that should be added to test water.

2. Influent water is prepared in accordance with relevant requirements. Two different salinities are chosen: 33-34 PSU and 22-23 PSU. The salinity of the raw water is adjusted by adding sea salt or fresh water; the concentration of DOC, POC and TSS can be adjusted by adding bottom sediment or even glucose and starch to raw water. Please refer to the following table for details:

Table 2-4 Preparation of raw water quality

Parameter	Method	Quantity	
Salinity	---	22-23	33-34
DOC	Required value (mg/L)	＞5	＞1
	Adding glucose(kg)	11.25	2. 25
POC	Required value (mg/L)	＞5	＞1
	Adding starch(kg)	10.12	2.02
TSS	Required value (mg/L)	＞50	＞1
	Adding bottom sediment (kg)	45.0	0.9
Note	The volume of raw water is supposed to be 900 m^3.		

2. Influent water indicator requirements:

a. G8 Requirements

The influent water should include:

.1 test organisms of greater than or equal to 50 micrometres or more in minimum dimension should be present in a total density of preferably 10^6 but not less than 10^5 individuals per cubic metre, and should consist of at least 5 species from at least 3 different phyla/divisions;

.2 test organisms greater than or equal to 10 micrometres and less than 50 micrometres in minimum dimension should be present in a total

density of preferably 10^4 but not less than 10^3 individuals per millilitre, and should consist of at least 5 species from at least 3 different phyla/divisions;

.3 heterotrophic bacteria should be present in a density of at least 10^4 living bacteria per millilitre; and

.4 the variety of organisms in the test water should be documented according to the size classes mentioned above regardless if natural

organism assemblages or cultured organisms were used to meet the density and organism variety requirements.

b. D-2 Regulation

Separate samples should be collected for:

.1 organisms of greater than or equal to 50 micrometres or more in minimum dimension;

.2 organisms greater than or equal to 10 micrometres and less than 50 micrometres in minimum dimension;

.3 for coliform, enterococcus group, *Vibrio cholerae* and heterotrophic bacteria;

Regulation D-2 stipulates that ships meeting the requirements of the Convention by meeting the ballast water performance standard must discharge:

.1 less than 10 viable organisms per cubic metre greater than or equal to 50 micrometres in minimum dimension;

.2 less than 10 viable organisms per millilitre less than 50 micrometres in minimum dimension and greater than or equal to 10 micrometres in minimum dimension; and

.3 less than the following concentrations of indicator microbes, as a human health standard:

.1 Toxicogenic *Vibrio cholerae* (serotypes O1 and O139) with less than 1 Colony Forming Unit (cfu) per 100 millilitres or less than 1 cfu

per 1 gramme (wet weight) of zooplankton samples;

.2 *Escherichia coli* less than 250 cfu per 100 millilitres; and

.3 Intestinal *Enterococci* less than 100 cfu per 100 millilitres.

Table2-5 maximum value of testing three indicate microorganism in treated water

Treated water		
Parameters	Unit	Regulation
Toxicogenic *Vibrio chlorerae*	< 1 CFU/100 mL or < 1 CFU/ g zooplankter wet weight	Serotype O1 and O139
Escherichia coli	<250 CFU/ 100 mL	
Intestinal Enterococci	<100 CFU/ 100 mL	

D-2 Regulation is considered as treated water discharged rule. As a result of many red tide algae are less than 10μm, and it is omit that in addition to microorganisms which less than 10μm. However, for organisms' max. density, this regulation is clear. From another point of view, to select a proper organism's size is still specified by academic discussion which is subjective. What's more, IMO gave the organism a feasibility definition which is "organism and any period of vital movement", but for a More scientific definition is "the organisms which could complete their life cycle, including reproduce (DNA copy)". On the condition of UV light, several organisms' cells may destroy in heredity, but these organisms which lose fertility are still alive in a certain period.

2.4.2.2 Raw water preparation

1. Salinity adjustment

High salinity water adjustment:

It’s requested that testing raw water salinity is equal or higher than 32PSU during high salinity testing. And it’s feasible to add sea salt to raw water to increase the salinity if it’s lower than 32 PSU. We used sea salt form Dalian salt pit which is the most consistent with the testing water.

Salinity adjustment: High salinity seawater is prepared in advance by adding sea salt to seawater, and then mixing them with bubble generator. Then raw water is added and mixed with high salinity seawater to increase its salinity.

Additive sea salt amount (raw water storage tank volume: 2000 m^3 and it’s assumed that the testing salinity is 34 PSU):

Additive amount= (34-the present seawater salinity) x 2000 m^3

It’s tested that the seawater from testing sea area is 31 PSU, then the additive sea salt amount is 6000 kg to reach high salinity requirement.

Low salinity water regulation:

It’s requested that testing raw water salinity is from 3 to 32PSU during low salinity testing. So we need to add fresh water to the seawater as per the seawater from testing sea area is 31 PSU.

Additive fresh water amount (raw water storage tank volume: 2000 m^3 and it’s assumed that the testing salinity is 22 PSU):

The raw seawater amount= 22/31x2000=1419 m^3

Additive amount= 2000-1419=581 m^3

2. DOC & POC adjustment

It’s requested that DOC and POC concentration are larger than 1mg/L during high salinity testing in accordance with D-2 regulation. It’s tested that testing sea area water’s DOC and POC concentration are reach the requirement basically and there is no need to add substance.

It’s requested that DOC concentration is larger than 5mg/L during low salinity testing in accordance with D-2 regulation. It’s feasible to add glucose 6-phosphate to raw water to increase DOC concentration.

Additive glucose 6-phosphate amount (the raw water storage tank volume: 2000 m^3) =5x2000x10^3x10^{-6}/40%=25kg

It’s tested that when seawater biomass reaches testing requirement, POC concentration will be accordance with testing requirement during high salinity testing and low salinity testing. So there is no need to add substance to adjust POC since the biomass of testing sea area water reached the testing requirement.

3. TSS Accommodation

According to add airing and selected sea bottom mud to raw water tanks to adjust TSS. Firstly, to dissolve sea bottom mud to 12 containers with 2m^3, then mixed. After adding muddy water to raw water tank, cycling with circulator pump.

TSS should up to 1mg/L in accordance with D-2, during high salinity cycling. The TSS of COSCO (Dalian) shipyard port is

above1mg/L. Therefore, needn't add any substance for accommodation.

TSS should up to 50mg/L in accordance with D-2, during mid salinity cycling. Add dry sea bottom mud to increase concentration of TSS to meet the raw water requirement.

When 2000m^3 raw water, the added weight of sea bottom mud by testing is as follow:

Weight of sea bottom mud=50x2000x10^3x10^{-6}=100kg

Testing result shows that about 70% of the sea bottom mud dissolve, Weight of added sea bottom mud =100/70%=143kg.

4. Accommodation of the organisms' type and density in raw water

Adjust the organisms' type by testing local organisms together with captive breeding. Ensure the add proportion in accordance with D-2 by matching test.

Fig.2-4 Organisms culturing room

Minimum size ≥50μm: through testing the local organisms which

size ≥50μm, there are many types of organisms, including copepoda, protozoon, planktonic larva, cllium and diatom etc. but quantity not sufficient. In order to meet G8, adding captive breeding *Karatella*(20%) and *Artemia salina*(60%) as the superiority species and the eggs were brought from Shandong.

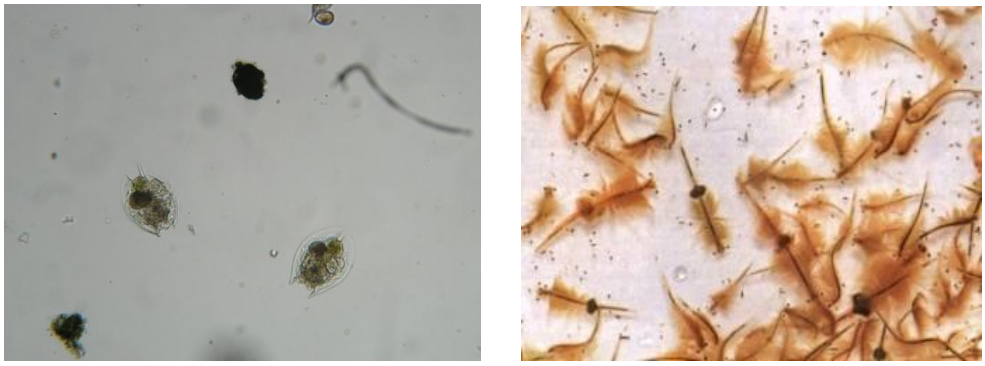


Fig.2-5 *Karatella* and *Artemia salina*

Phytoplankton with minimum size ≥10~50μm: Through culturing *Isocrysis galbana, Dunaliella salina, Platymanas helgolandica* and *Cylindrotheca costerium* to control the organisms' density up to 10^6cells/ml. Adding proportion is 1:500 by calculation. There are 30% *Isocrysis galbana,* 20% *Dunaliella salina*, 15% *Platymanas helgolandica* and 20% *Cylindrotheca costerium* in raw water. The diatom is the main phytoplankton in local area, the dominant species are *skeletonema costatum, Coscinodiscus, Thalassiosira* and *Chaetoceros.* After adding these culturing algae, the phytoplankton's type and density could meet G8 requirement.

Through adding beef extract and peptone into sea water which take from local area and expansion foster indoors to make the heterotrophic

bacteria density up to 10^9cells/ml. Add water mass experiment consumption ratio for 1:100000.

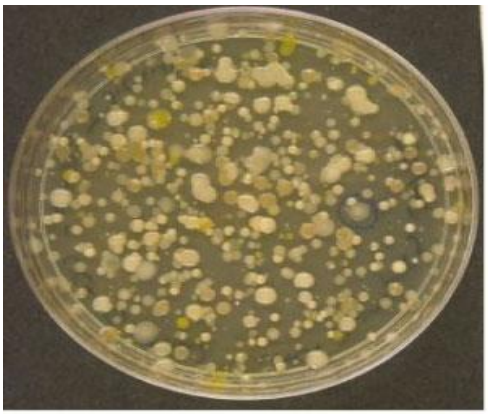


Fig.2-6 bacterial clump

2.5 Testing program

Land-based test is conducted from Mar. 2012. Two salinities testing are set, they are high salinity testing with water salinity 34 PSU and low salinity testing with water salinity 22 PSU. Every five cycles are done during each salinity testing.

There are three types tank: raw water tank, ballast tank and control tank. Raw water is prepared to reach D-2 standard and storage in raw water tank. Raw water is pumped to filter and then flows into EPT (enhanced physical treatment) unit, then the treated water flows to ballast tank. Water is sampled before and after it flows to ballast water management system. At the same time, water from raw water tank is pumped to control tank directly. After 120 h storage in ballast tank and control tank, water is discharged. Discharged water is sampled to determine water quality and biomass. The testing arrangement schedule

is as following tables.

Table 2-6 Time schedule

2012-03-16	Preparation for H1 ballasting testing
2012-03-17	H1 ballasting testing
2012-03-18	Preparation for H2 ballasting testing
	H2 ballasting testing
2012-03-19	Preparation for H3 and H4 ballasting testing
	H3 and H4 ballasting testing
2012-03-20	Preparation for H5 and L1 ballasting testing
	H5 and L1 ballasting testing
2012-03-21-	Preparation for L2 and L3 ballasting testing
	L2 and L3 ballasting testing
2012-03-22	Preparation for L4 and L5 ballasting testing
	L4 and L5 ballasting testing
2012-03-22	H1 de-ballasting testing
2012-03-23	H2 de-ballasting testing
2012-03-24	H3 and H4 de-ballasting testing
2012-03-25	H5 and L1 de-ballasting testing
2012-03-26	L2 and L3 de-ballasting testing
2012-03-27	L4 and L5 de-ballasting testing

Table 2-7 Detailed information of testing progress

Testing items	Date	Time	Duration(min)	Sampling time
H1 ballasting testing (treatment)	2012-03-17	21:28-22:40	72	21:40
H1 ballasting testing (control)		22:42-23:52	70	22:45
H2 ballasting testing (treatment)	2012-03-18	0:11-1:33	82	00:25
H2 ballasting testing (control)		1:39-2:54	75	01:48
H3 ballasting testing (treatment)	2012-03-19	16:10-17:32	82	16:20
H3 ballasting testing (control)		17:46-19:06	80	17:55
H4 ballasting testing (treatment)	2012-03-19	20:15-21:37	82	20:25
H4 ballasting testing (control)		21:40-23:00	80	21:50
H5 ballasting testing (treatment)	2012-03-20	15:28-16:51	83	15:40

H5 ballasting testing (control)		17:05-18:25	70	17:15
L1 ballasting testing (treatment)	2012-03-20	21:00-22:20	80	21:10
L1 ballasting testing (control)		22:24-23:34	70	22:30
L2 ballasting testing (treatment)	2012-03-21	23:49- 1:01	72	00:05
L2 ballasting testing (control)		1:02-2:11	69	01:15
L3 ballasting testing (treatment)	2012-03-21	19:48-21:11	83	20:00
L3 ballasting testing (control)		21:17-22:37	80	21:27
L4 ballasting testing (treatment)	2012-03-21	22:40- 0:03	83	22:53
L4 ballasting testing (control)	2012-03-22	0:10-1:30	80	00:20
L5 ballasting testing (treatment)	2012-03-22	1:40-3:03	83	01:50
L5 ballasting testing (control)		3:40-5:00	80	03:50
H1 de-ballasting testing (treatment)	2012-03-23	19:08-20:01	53	19:15
H1 de-ballasting testing (control)		20:37-21:26	49	20:40
H2 de-ballasting testing (treatment)	2012-03-23	22:57-23:51	54	23:05
H2 de-ballasting testing (control)	2012-03-24	0:17-1:06	49	00:20
H3 de-ballasting testing (treatment)	2012-03-24	17:38-18:26	48	17:40
H3 de-ballasting testing (control)		19:25-20:16	51	19:30
H4 de-ballasting testing (treatment)	2012-03-24	22:01-22:50	49	22:05
H4 de-ballasting testing (control)		23:05-23:56	51	23:10
H5 de-ballasting testing (treatment)	2012-03-25	18:35-19:30	55	18:43
H5 de-ballasting testing (control)		19:38-20:25	47	19:40
L1 de-ballasting testing	2012-03-25	23:15-0:05	50	23:20

(treatment)				
L1 de-ballasting testing (control)	2012-03-26	0:18-1:08	50	00:20
L2 de-ballasting testing (treatment)	2012-03-26	2:03-2:55	52	02:10
L2 de-ballasting testing (control)		4:15-5:05	50	04:20
L3 de-ballasting testing (treatment)	2012-03-26	22:04-23:01	57	22:15
L3 de-ballasting testing (control)		23:17-0:05	48	23:20
L4 de-ballasting testing (treatment)	2012-03-27	0:20-1:10	50	00:25
L4 de-ballasting testing (control)		1:50-2:42	47	01:55
L5 de-ballasting testing (treatment)	2012-03-27	3:25-4:20	55	03:35
L5 de-ballasting testing (control)		6:10-6:40	30	06:14

2.6 Sampling and Analysis

2.6.1 Sampling

In order to sample in an orderly way, 9 dedicated container with six 50L and three 1.5m^3 would be prepared. When sampling, all the samples were collected in 50L container through sampling point 1 and 1.5m^3 through sampling point 2 and 3, then divided into different containers for testing different parametric.

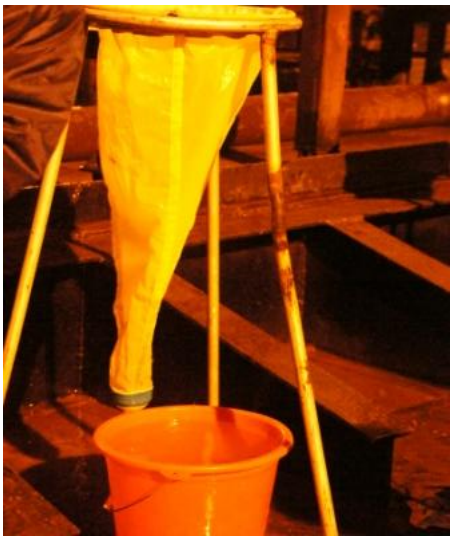
In addition to DO, all the water parameters were tested in a 5L plastic drum sampling directly from the outlet. After mixing, took samples to test and pretreatment from it in the field laboratory. To test DO, a latex tubing with a sampling pipe which was specialized suited to

ballast water imitate container would be applied to siphon the samples to brown glass bottles.

≥50μm organisms were filtered with a 50cm diameter 1m length (Fig2-8 a) net which was hold by steel frame and on the dewatering outlet of the big plastic drum (Fig2-7). Then collected the samples in the container at the bottom of the net to a little plastic durm posted label. 10~50 μm organisms were filtered with a 20cm diameter 1m length (Fig2-8 b) net, and filtered 5 times with a 2L measuring pot. Then collected the samples in the container at the bottom of the net to a little plastic durm posted label.

Microorganism samples were sampled directly at the discharge outlet to prevent polluted by the microorganism in the air. Sterilized by high temperature before sample, and wore disposable glove during sampling to achieve aseptic operation.

Fig2-7 sampling drum



a 50μm net b 10μm net

Fig.2-8 Sampling drum and filter net

2.6.2 Sample retention and transportation

2.6.2.1 Testing items

Water quality items: temperature, salinity, pH, DO, NTU, DOC, POC, TSS.

Organism items: organisms which minimum size is equal or larger than 50 μm and organisms which minimum size is equal or larger than 10μm and smaller than 50μm.

Microorganism items: heterotrophic bacterium, Toxicogenic Vibrio cholerae, Escherichia coli, Intestinal Enterococci.

2.6.2.2 Sample retention and transportation

Temperature, salinity and NTU are monitored on site, the other items are required to be saved and then monitored in laboratory.

Chemical items samples, bacterium, organisms which minimum size is equal or larger than 50 μm and organisms which minimum size is equal or larger than 10μm and smaller than 50μm are sampled separately and saved in different sampling bottles with labels. Seal up these bottles, save them in incubator and then transport the samples to laboratory and pre-treat them in 2 hours. Conventional physicochemical index and biological index samples retention and pretreatment are done by professional staff. A special lab is built in Dalian (COSCO) Shipyard to make sure all the samples are tested or pre-treated in 6 hours after sampled.TSS, POC and DOC samples is saved in incubator in frozen condition on passage, and then saved in -20℃ refrigerator in lab in QINGDAO. Biological samples are counted immediately after they are sampled, and then are fixed (organisms which minimum size is equal or larger than 50 μm is fixed with formaldehyde and organisms which minimum size is equal or larger than 10μm and smaller than 50μm is fixed with lugol's iodine solution), then the fixed samples are sent to lab to identify and count.

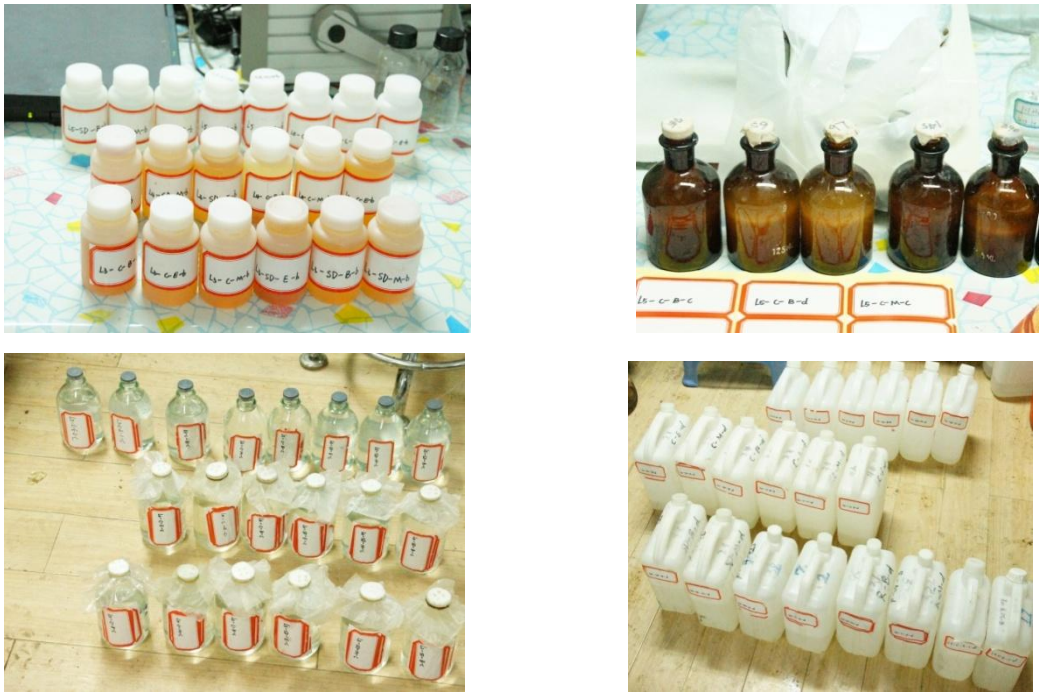


Fig.2-9 samples

2.6.3 Testing Item

2.6.3.1 Testing of Environmental and Biological Parameters

According to the requirements of the project and G8 guidelines, the following parameters should be tested:

1) Environmental parameters: temperature, salinity, pH, DO, turbidity, DOC, POC, TSS; Moreover, the UV transmittance will be tested in the land-based testing.

2) Biological parameters: organisms for minimum dimension greater than 50 μm (mainly for plankton), and organisms for minimum dimension between 10 and 50 μm (mainly for phytoplankton), *Heterotrophic bacteria*, *Escherichia coli*, *Intestinal Enterococci* and *Vibrio cholera*.

Details of testing methods are shown in Table 2-8:

Table 2-8 Testing Methods

Parameter	Pre-treatment	State	Storage condition	Maximum holding time	Instrument
Temperature	Immediately determined	/	/	/	Multi-parameter probe
Salinity	Immediately determined	/	/	/	Multi-parameter probe
NTU	Immediately determined after sampling	/	/	/	Spectrophotometer.
pH	Immediately determined after sampling	/	/	/	pH meter.
DO	Immediately fixed	/	/	/	Iodometry method
TSS	Directly filtered	Filtered membrane	-20 °C	72 h	Electronic balance
POC	Directly filtered	Filtered membrane	-20 °C	72 h	ElementarVarioELIII, German
DOC	Directly filtered	Filtered water	-20 °C	72 h	TOC-VcpH analyzer ,Japan
UV transmittance	/	Water	-20 °C	72 h	UV spectrophotometer
>50 μm organisms	Stained by neutral red after sampling	Filtered water	-20 °C, no light	72 h	Counted and observed by stereo microscope
10~50 μm organisms	/	Filtered water	/	6 h	PAM fluorometer
10~50 μm organisms	Immediately fixed by Lugol lodine solution after sampling, then dyed by FDA-PI after sampling	Filtered water	4-6 °C	72 h	Stained by FDA-PI; and then Counted and observed by inverted fluorescence microscope
Heterotrophic bacteria	Immediately inoculated within 2 hours after sampling	Plate method	25 °C	7 d	Counting the number of colonies by eye
Vibrio cholera	Immediately inoculated within 2 hours after sampling	Plate method	37 °C	18 h	Counting the number of colonies by eye
Escherichia coli	Immediately inoculated within 2 hours after sampling	Filter membrane method	44 °C	18-24 h	Counting the number of colonies by eye
Intestinal enterococci	Immediately inoculated within 2 hours after sampling	Filter membrane method	37 °C	24 h	Counting the number of colonies by eye

Among these, details for testing organisms are:

1) Organisms for minimum dimension greater than 50 μm:

Water samples will be filtered through 50 μm (in the diagonal dimension) silk screen (sample volume is determined according to the density of plankton, generally 500~1000L at least); the collected organisms will be directly observed and counted with a stereomicroscope. If it is hard to count the organisms directly, observe the activity of the organisms with a microscope and make records firstly, and then samples will be stained by neutral red and stored at -20 °C. When transported to the laboratory, samples will be defrosted firstly and the numbers of the organisms will be counted according to the degree of color.

$$C_B = \frac{N_B}{V}$$

Where:

C_B — quantity of plankton in every cubic meter, ind/m^3;

N_B — quantity of plankton filtered by the screen, ind or cells;

V — volume of filtered ballast water, m^3.

2) Organisms for minimum dimension between 10 and 50 μm:

Water samples will be filtered through 10 μm (in the diagonal dimension) silk screen; the volume will be decided according to cell concentration of plankton, and at least 0.5~2 L sample will been taken generally; the activity of the organisms will first be observed by using an inverted microscope and then 1% Lugol lodine solution will be added to

fix organisms. After that, samples will be stored with no light and transported to laboratory. In the laboratory, FDA will first be added to the sample; after fully mixing PI will be added to the sample. Under blue light (Max wavelength: 495 nm), alive cells are stained to be bright green and dead cells are stained to be red. Photographs of staining degree will be taken by Nikon camera. Quantity of organisms will be observed and counted by using fluorescence microscope.

3) *Heterotrophic bacteria*: plate counting method

Principle: a single bacteria cell can form a visible colony when it is incubated at the plating medium for a certain time. That means a colony represents a bacteria cell so that you the numbers of bacteria cells can be calculated through the colonies. The key of counting is that all bacteria must be divided completely, and the samples should be diluted into even different concentrations and inoculated on the culture vessel with solid medium.

Method: 1 ml sample will be added into 100 ml water sample and mixed completely to divide the bacteria in aseptic conditions. Three series of 10-fold dilutions will be performed and 0.1 mL of each dilution will be plated on the culture vessel with solid medium (2216E). All plates will be incubated at 25 ℃ for 7 days, and the numbers of colonies on the plates will be counted.

4) *Vibrio cholera*: plate counting method

Vibrio cholera is an important parameter that can reflect water pollution levels leaded by the pathogenic microorganisms. The samples will be inoculated into TCBS flat selective medium and incubated for a certain time. Then the numbers of the colonies that have the characteristics of *vibrio* will be counted.

Method: Three different concentrations of water samples will be inoculated into BTB culture solution and incubated at 37 °C for 18 h. The bacteria solution will be divided on the TCBS flat plate, and all plates will be incubated at 37 °C for 18 h. Then the green, blue-green and yellow colonies will be inoculated into CPA medium slant. The numbers of the colonies that have the characteristics of *Vibrio cholera* will be counted and calculated according to Sheet MPN.

5) *Escherichia coli*: filter membrane method

The water sample will be put into aseptic filter and extracted through micro filtration membrane in the filter. The membrane will be stick into appropriate selective medium and incubated for a certain time. Then the numbers of the colonies on the membrane will be counted and calculated.

Method: The water sample will be put into aseptic filter and extracted through micro filtration membrane (acetate fiber membrane with 0.2 μm aperture) in the filter. The membrane will be stick into appropriate selective medium (M-TEC) and incubated at 37 °C for 0.5 h

and then at 44 °C for 18~24 h. Then the numbers of the colonies on the membrane will be counted and calculated.

6) *Intestinal Enterococci*: filter membrane method

The sample will be inoculated into PSE AGAR plate selective medium and incubated at 37 °C for a certain time. Then the numbers of the colonies that have the characteristics of *enterrococcus* will be counted. The method is the same as that for *Escherichia coli* analysis.

2.7.3.3 Instruments

Table 2-9 Instruments

Number	Name	Specification	Precision	Producer
1	Filter mambrane	Membrane diameter 25cm	Bore diameter 50μm	America
2	Filter mambrane	Membrane diameter 25cm	Bore diameter 10μm	America
3	pH meter	PHS-3C	0～14, 0.01pH	Shanghai, China
4	Electronic balance	ME614S	0～610g, 0.1mg	Germany
5	TOC analysis meter	TOC--5000	0—2500mg/L, <1.5%	Japan
6	POC analysis meter	ElementarVarioELIII	±0.2%	Germany
7	Fluorescence microscope	EC501	100—1000×	Japan
8	Inverted fluorescence microscope	TE2000-U	40—400×	Japan
9	Inverted microscope	TS100	40—400×	Japan
10	Anatomical lens		10—200×	Opten, West Germany
11	Microcomputer counter	MCC1-1	100×3 10 60 100	Qingdao, China
12	Filter device	250ml, 500ml		Qingdao, China
13	Multiparameter analyzer	SG3	Temperature 0.0-100.0, ±0.1°C Salinity 0.00—80.00, ±0.5%	Switzerland

3 CONSEQUENCES

3.1 Temperature and Salinity

It is between the end of winter and the beginning of spring to do the testing. The air and the water temperature is around 1.5 ~ 3.1℃. Because adjust the salinity in one tank, the salinity fluctuation among cycles is little. High Salinity Cycles are 33.3~33.6 PSU, Low Salinity Cycles are 22.4~22.6 PSU.

Table 3-1 Temperature & Salinity in land-based testing

High Salinity Cycles（>32PSU）		Salinity （PSU）		Temperature（ T℃）	
		AVG.	SD	AVG.	SD
Ⅰ	Ballast（D0）	33.3	0.07	1.6	0.10
	Deballast（D5）	33.4	0.06	2.1	0.04
Ⅱ	Ballast（D0）	33.4	0.08	1.5	0.09
	Deballast（D5）	33.5	0.11	2.2	0.05
Ⅲ	Ballast（D0）	33.5	0.07	2.1	0.07
	Deballast（D5）	33.5	0.03	2.7	0.06
Ⅳ	Ballast（D0）	33.6	0.04	2.6	0.07
	Deballast（D5）	33.5	0.11	2.7	0.05
Ⅴ	Ballast（D0）	33.3	0.06	2.6	0.05
	Deballast（D5）	33.4	0.06	3.0	0.06
AVG.		33.4		2.3	
Low Salinity Cycles（22PSU）					
Ⅰ	Ballast（D0）	22.4	0.08	2.5	0.05
	Deballast（D5）	22.5	0.11	2.7	0.05
Ⅱ	Ballast（D0）	22.4	0.06	2.6	0.08
	Deballast（D5）	22.5	0.14	2.7	0.06
Ⅲ	Ballast（D0）	22.4	0.08	2.7	0.04
	Deballast（D5）	22.4	0.14	2.8	0.03
Ⅳ	Ballast（D0）	22.5	0.06	2.8	0.05
	Deballast（D5）	22.4	0.06	3.0	0.05
Ⅴ	Ballast（D0）	22.6	0.06	2.9	0.05
	Deballast（D5）	22.4	0.08	3.1	0.05
AVG.		22.5		2.7	

3.2 Water quality

3.2.1 pH & DO

pH of high salinity: The pH variation range is 8.15~8.30 mg/L, average in 8.24mg/L. The average pH value of raw water, control inflow water and control discharged water are are 8.26 mg/L, 8.27 mg/L and 8.26mg/L separately which are a little higher than treated ballast and deballasting water.

pH of low salinity: The average pH value is 8.01 mg/L. There has a same trend with high salinity.

Table 3-2 Water quality of high salinity

>32PSU	Water type	pH		NTU		TSS(mg/L)		DO(mg/L)		DOC(mg/L)		POC(mg/L)	
		Avg.	sd	Avg.	sd	Avg.	sd	Avg.	sd	Avg.	sd	Avg.	sd
Ⅰ	Control inflow water	8.26	0.01	4.64	0.22	1.87	0.07	9.02	0.05	1.60	0.08	1.14	0.02
	Treated inflow water	8.23	0.01	3.70	0.32	1.34	0.12	8.70	0.05	1.02	0.06	0.70	0.09
	Raw water	8.25	0.01	4.61	0.22	1.87	0.09	9.02	0.13	1.47	0.04	1.10	0.16
	Treated discharged water	8.17	0.02	2.82	0.60	1.00	0.07	8.78	0.03	0.40	0.04	0.34	0.07
	Control discharged water	8.24	0.01	4.20	0.14	1.65	0.05	8.83	0.01	0.96	0.13	0.86	0.11
Ⅱ	Control inflow water	8.27	0.01	4.66	0.50	1.78	0.04	9.03	0.08	1.11	0.07	1.09	0.02
	Treated inflow water	8.20	0.01	3.51	0.42	1.52	0.16	8.95	0.15	0.69	0.10	0.55	0.12
	Raw water	8.25	0.01	4.49	0.25	1.82	0.03	9.01	0.06	1.32	0.12	1.15	0.12
	Treated discharged water	8.24	0.01	2.09	0.30	1.14	0.09	8.85	0.09	0.69	0.17	0.38	0.10
	Control discharged water	8.25	0.01	3.18	0.24	1.54	0.11	8.93	0.06	1.22	0.13	0.55	0.17
Ⅲ	Control inflow	8.27	0.01	4.69	0.10	1.75	0.15	8.94	0.05	1.46	0.13	1.30	0.16

	water												
	Treated inflow water	8.16	0.01	3.20	0.10	1.29	0.06	8.56	0.12	1.17	0.03	0.99	0.20
	Raw water	8.27	0.01	4.87	0.45	1.69	0.11	8.93	0.04	1.53	0.02	1.22	0.05
	Treated discharged water	8.20	0.01	2.06	0.07	0.93	0.10	8.69	0.09	0.66	0.14	0.49	0.10
	Control discharged water	8.25	0.01	3.91	0.27	1.59	0.13	8.89	0.06	1.18	0.10	1.14	0.19
Ⅳ	Control inflow water	8.29	0.01	4.93	0.75	1.81	0.27	9.04	0.11	1.46	0.06	1.15	0.05
	Treated inflow water	8.21	0.01	3.79	0.11	1.41	0.21	8.76	0.14	1.10	0.09	0.93	0.13
	Raw water	8.27	0.02	5.13	0.51	1.78	0.20	8.99	0.11	1.42	0.08	1.19	0.02
	Treated discharged water	8.19	0.01	2.25	0.20	1.01	0.03	8.82	0.04	0.77	0.15	0.43	0.08
	Control discharged water	8.28	0.01	3.56	0.37	1.62	0.17	8.95	0.11	1.21	0.04	0.67	0.09
Ⅴ	Control inflow water	8.29	0.01	5.22	0.83	1.73	0.15	9.06	0.09	1.33	0.08	1.25	0.07
	Treated inflow water	8.21	0.01	3.32	0.33	1.16	0.06	8.94	0.07	1.01	0.13	0.89	0.16
	Raw water	8.28	0.01	5.17	0.55	1.71	0.08	9.02	0.14	1.24	0.18	1.32	0.09
	Treated discharged water	8.19	0.01	1.84	0.16	0.89	0.08	8.80	0.02	0.72	0.13	0.38	0.10
	Control discharged water	8.25	0.01	2.61	0.25	1.45	0.13	8.96	0.04	1.30	0.19	1.01	0.18
Avg.	Control inflow water	8.27		4.83		1.79		9.02		1.39		1.185	
	Treated inflow water	8.20		3.50		1.34		8.78		1.00		0.812	
	Raw water	8.26		4.85		1.77		8.99		1.40		1.196	
	Treated discharged water	8.20		2.21		0.91		8.79		0.65		0.403	
	Control discharged water	8.26		3.49		1.57		8.91		1.18		0.844	

DO of high salinity: The DO variation range is 8.22~9.18 mg/L. The average DO value of raw water is almost same as control inflow water. They are 9.02 mg/L and 8.99 mg/L separately, and most samples DO value are around 9 mg/L. While the control discharged water average DO value is 8.91 mg/L which is a little lower than raw water. The value of treated inflow water is 8.78 mg/L which is close to the DO value of treated discharged water (8.79 mg/L).

DO of low salinity: The variation range of low salinity is 7.58mg/L~8.28mg/L. The value of raw water and control inflow water is almost same(8.22 mg/L), but a little drop for control discharged water (8.15 mg/L). The value of treated inflow water and treated discharged water is 7.79 mg/L and 7.64 mg/L separately.

Table 3-3 Water quality of low salinity water

<22PSU	Water type	pH		NTU		TSS(mg/L)		DO(mg/L)		DOC(mg/L)		POC(mg/L)	
		Avg.	sd	Avg.	sd	Avg.	sd	Avg.	sd	Avg.	sd	Avg.	sd
I	Control inflow water	8.04	0.02	13.20	0.87	50.60	2.17	8.28	0.03	5.18	0.04	5.18	0.07
	Treated inflow water	7.99	0.01	8.82	0.54	21.27	0.67	7.71	0.09	3.71	0.41	2.80	0.73
	Raw water	8.04	0.02	13.18	0.39	50.94	0.63	8.25	0.01	5.38	0.28	5.21	0.22
	Treated discharged water	7.91	0.01	5.32	0.56	16.99	0.93	7.78	0.18	3.29	0.20	2.42	0.22
	Control discharged water	8.05	0.01	9.42	0.34	32.28	1.81	7.83	0.16	4.22	0.17	3.26	0.11
II	Control inflow water	8.03	0.11	13.86	0.52	52.76	4.12	8.11	0.23	5.62	0.28	5.21	0.43
	Treated inflow water	7.87	0.07	8.14	1.02	19.84	0.85	7.89	0.15	3.81	0.22	4.25	0.33
	Raw water	7.97	0.01	14.17	2.20	53.30	2.69	8.12	0.14	5.47	0.31	5.19	0.16
	Treated discharged water	7.85	0.03	5.34	0.66	16.73	0.80	7.67	0.26	3.80	0.25	3.07	0.11
	Control discharged	8.04	0.01	9.87	0.74	31.11	1.11	8.24	0.37	4.78	0.30	3.53	0.25

	water												
Ⅲ	Control inflow water	8.03	0.03	13.56	1.11	50.73	0.69	8.22	0.21	5.43	0.29	5.20	0.18
	Treated inflow water	7.99	0.01	8.83	0.52	21.20	1.89	7.89	0.28	4.41	0.24	3.74	0.37
	Raw water	8.01	0.10	13.40	1.03	50.33	0.71	8.23	0.19	5.54	0.29	5.25	0.15
	Treated discharged water	7.96	0.07	5.03	0.33	16.15	1.74	7.71	0.22	3.32	0.20	2.13	0.10
	Control discharged water	8.05	0.03	9.61	0.40	32.27	2.10	8.20	0.19	4.07	0.44	3.34	0.26
Ⅳ	Control inflow water	8.03	0.01	13.50	1.75	49.97	0.95	8.27	0.15	5.38	0.26	5.18	0.14
	Treated inflow water	7.94	0.05	7.85	1.02	22.27	3.04	7.84	0.29	4.88	0.06	3.25	0.49
	Raw water	7.99	0.08	11.39	0.80	50.23	1.61	8.23	0.04	5.02	0.21	5.04	0.27
	Treated discharged water	8.02	0.03	5.62	0.47	1684	0.80	7.58	0.12	3.44	0.38	2.81	0.49
	Control discharged water	8.05	0.01	10.94	0.57	32.39	2.42	8.27	0.05	4.15	0.15	3.16	0.23
Ⅴ	Control inflow water	8.02	0.03	12.11	0.30	50.19	1.13	8.19	0.12	5.28	0.31	5.11	0.03
	Treated inflow water	8.04	0.02	6.71	0.04	20.61	1.90	7.59	0.07	4.41	0.32	3.52	0.17
	Raw water	8.01	0.04	11.75	1.08	51.10	5.10	8.25	0.09	5.25	0.29	5.12	0.08
	Treated discharged water	8.03	0.00	5.06	0.23	14.71	1.08	7.80	0.23	3.19	0.04	2.12	0.07
	Control discharged water	8.05	0.02	9.85	0.48	24.31	0.88	8.20	0.20	3.74	0.31	3.09	0.33
Avg.	Control inflow water	8.03		13.24		50.85		8.21		5.38		5.18	
	Treated inflow water	7.99		8.07		21.04		7.79		4.24		3.51	
	Raw water	8.00		12.78		51.18		8.22		5.33		5.16	
	Treated discharged water	7.98		5.28		16.28		7.64		3.41		2.51	
	Control discharged water	8.05		9.94		30.47		8.15		4.19		3.28	

3.1.2 Turbidity & TSS

Turbidity of high salinity:The turbidity variation range of raw water and control inflow water of high salinity is 4.49~5.55, and average is 4.84. After 5 days storage, the turbidity of control discharged water is3.49, lower than inflow water. The turbidity of inflow water and discharged water in treated tank is 3.50 and 2.21 separately.

Turbidity of low salinity: The turbidity is almost three times as high salinity. The average turbidity of raw water, control inflow water, control discharged water and treated discharged water is 13.24, 12.78, 9.94 and 5.28 separately.

TSS of high salinity: The TSS concentration of raw water is almost same as control inflow water, the variation range is 1.528~2.060 mg/L, average is 1.78 mg/L. And the treated inflow water and discharged water TSS concentration are 1.34 mg/L and 0.91 mg/L.

TSS of low salinity: The variation range of TSS concentration is large, TSS concentration of raw water, control inflow water, treated inflow water and treated discharged water are 50.85 mg/L, 51.18 mg/L, 21.04 mg/L and 16.28 mg/L separately.

3.2.2 DOC & POC

The DOC concentration range of inflow water is between 1.03~1.69 mg/L in high salinity, while 4.79~5.92 mg/l in low salinity, the average

are 1.39mg/l and 5.35mg/L separately, both meets G8 land-based testing requirement. The density range of POC inflow water is between 0.95~1.40 mg/l in high salinity, and 4.74~5.56 mg/l in low salinity, the average is 1.17mg/l in high salinity and 5.17mg/l in low salinity, also meets G8 land-based testing requirement.

3.3 ≥50μm organisms

Organism of greater than or equal to 50μm is less in winter in the testing sea area. Main types are Calanis sinicus, Oithona sp., Tridentiger larvae and larger diatom Thalassiosira spp., Coscinodiscus spp., and Protozoa etc.. Besides, add Artenia salina and Brachionus sp. Which are cultivated on spot, so the organism species greater than or equal to 50μm can meet the requirement of G8.

Organism of the size-range mainly are Thalassiosira spp. In diatom and added Artenia salina and Brachionus. Quantity details in each cycle are listed in Table 3.4., and there is no big difference in quantity and density of the two salinity raw water and control inflow water, high salinity in 1.67×105 ind./m3 and 1.70×105 ind./m3, and low salinity in 1.66×105 ind./m3 and 1.64×105 ind./m3. The organism quantity in deballasting water lower two degree after 5 days later. The cultured Artenia salina and Brachionus sp. quantity decreases largely. After flowing through the filter(before into the process unit), the inflow water

density lower obviously, including the dead organisms, and the total density fluctuates between 10~102 ind./m3. The average living organism density (T-0) of high and low salinity is 12.4 ind./m3 and 13.20 ind./m3. 5 days later, no living organism is detected in high and low salinity in the second treatment seawater.

Table3-4 Total density of ≥50 μm organisms in land-based testing（ind /m^3）

High salinity	Control tank						Treated tank		
	Raw water（R-0）		Discharged water（C-5）		Inflow water（RC-0）		Inflow water（T-0）		Discharged water（T-5）
	Average	sd	Average	sd	Average	sd	Average	sd	Average
I	1.14E+05	6.89E+03	6.97E+03	9.68E+02	1.10E+05	3.53E+03	13.0	10.0	No living organisms
II	1.34E+05	4.71E+03	4.11E+03	5.45E+02	1.28E+05	2.90E+03	14.7	1.15	No living organisms
III	1.99E+05	5.58E+03	4.37E+03	3.70E+02	2.19E+05	6.44E+03	8.3	3.21	No living organisms
IV	2.08E+05	2.24E+04	1.81E+03	2.09E+02	1.94E+05	1.92E+04	10.7	7.23	No living organisms
V	1.82E+05	1.26E+04	3.01E+03	75.9	1.98E+05	1.20E+04	15.3	2.08	No living organisms
Average	1.67E+05		4.05E+03		1.70E+05		12.4		No living organisms
Low salinity									
I	1.81E+05	1.12E+04	1.40E+03	1.85E+02	1.84E+05	4.41E+03	11.0	2.00	No living organisms
II	1.67E+05	2.11E+03	1.15+E03	61.0	1.46E+05	7.38E+03	13.3	1.15	No living organisms
III	1.56E+05	3.18E+04	2.71E+03	3.1E+02	1.66E+05	2.80E+03	16.3	1.15	No living organisms
IV	1.62E+05	1.24E+04	1.72E+03	2.02E+02	1.67E+05	2.90E+03	10.3	2.31	No living organisms
V	1.66E+05	6.04E+03	2.31E+03	59.6	1.59E+05	6.67E+03	15.3	3.21	No living organisms
Average	1.66E+05		1.86E+03		1.64E+05		13.2		No living organisms

3.4 10~50μm organisms

The type and density is less for 10~50μm organisms in biocoenosis. The normal types are Thalassiosira spp、*Skeletonema costatum*、*Rhizosolenia delicatula*、*Ditylum brightwellii* and *Coscinodiscus* spp.. In order to make the raw water density reach to 10^3cell/mL, and get dominant species, adds 4 kinds of algae to cultivate in each testing cycle: *Isochrysis galbana*、*Platymonas helgolandica*, *Dunaliella salina* and *Cylindrotheca closterium*.

Table.3-5 Total density of 10~50 μm organisms in land-based testing（cell/mL）

total density of 10~50 μm organisms					
High salinity	Raw water（R-0）	Control tank		Treated tank	
		Inflow water（RC-0）	Discharged water（C-5）	Inflow water（T-0）	Discharged water（T-5）
I	1018.2±9.6	1010.1±18.9	121.8±7.3	4.8±0.3	No living organisms
II	1156.6±100.6	1172.0±55.8	101.8±4.9	8.3±1.0	No living organisms
III	1134.7±55.1	1152.5±61.4	142.4±4.6	4.9±0.3	0.004±0.003
IV	1166.6±36.9	1175.4±43.8	125.1±5.8	6.6±0.4	0.002
V	1213.5±64.5	1152.8±44.3	108.9±9.0	9.9±1.2	0.002
Average	1137.9	1132.5	120.0	6.9	
Low salinity	Raw water（R-0）	Control tank		Treated tank	
		Inflow water（RC-0）	Discharged water（C-5）	Inflow water（T-0）	Discharged water（T-5）
I	1213.9±33.6	1225.7±40.1	129.4±3.7	4.5±0.7	No living organisms
II	1114.5±38.8	1105.0±20.9	124.5±7.5	4.1±0.4	No living organisms
III	1101.7±15.3	1128.2±14.1	116.8±5.9	3.6±0.3	No living organisms
IV	1119.9±5.7	1125.1±34.3	119.7±4.1	3.5±0.4	No living organisms
V	1016.3±24.1	1018.2±11.9	106.6±3.0	3.5±0.3	No living organisms
Average	1113.3	1120.5	119.4	3.8	

3.5 Phytoplankton Photosynthetic Activity

Phytoplankton photosynthesis capacity (also known as photosynthetic activity, Fv / Fm) is an effective indicator to reflect the physiological status of phytoplankton. The principle is that chlorophyll fluorescence signal generated within the algal cells contains very rich information about photosynthesis characteristics while it is very easy to change with the environmental conditions. When fluorescent is absorbed by chlorophyll molecules, the chlorophyll molecule transits from the ground state to the excited state; as the excited state is very unstable, the chlorophyll molecule will release the energy and go back to the ground state, which is known as fluorescence phenomenon. Phytoplankton pulse amplitude modulated fluorometer (PAM) which is designed based on the principle of chlorophyll fluorescence can provide a lot of information about complex photosynthesis process, including the absorption of light quanta, energy conversion, and has been efficiently used to study plant photosynthesis.

During this land-based test, Fv / Fm was only analyzed for discharged water samples after 5 days store in each testing cycle. From Table 3.6 it was demonstrated that Fv / Fm of control tank with high salinity was not high. Its average was 0.27 and fluctuations range was from 0.12 to 0.39. In particular, it was a little low for testing cycle 1 and 2 whose value was 0.2. This may be caused by the cold air on that testing

day. The cultured algae inactivated or even died due to low temperature. The Fv / Fm of group with low salinity group was a little higher and the average was 0.33. According to some existed testing results, the Fv / Fm of inflow water is generally above 0.5 or more, while that of natural sea area or single cultured algae was a little higher. It was deduced that cold water temperature was the main reason that caused low Fv / Fm. The Fv / Fm of water samples from treated tank decreased below 0.1 and generally was between 0.03 to 0.07.

Table 3.6 Fv/Fm of discharged water sample after 5 days store

High Salinity	Control Tank C-5		Treated Tank T-5	
	AVG	SD	AVG	SD
I	0.21	0.07	0.09	0.05
II	0.20	0.02	0.06	0.02
III	0.28	0.07	0.07	0.02
IV	0.32	0.02	0.04	0.01
V	0.32	0.02	0.04	0.01
AVG	0.27		0.06	
Low Salinity				
I	0.34	0.01	0.04	0.01
II	0.34	0.01	0.03	0.01
III	0.32	0.02	0.04	0.01
IV	0.33	0.01	0.05	0.01
V	0.32	0.02	0.05	0.01
AVG	0.33		0.04	

3.6 Heterotrophic Bacteria

This land-based testing was performed at the end of winter, the quantity of heterotrophic bacteria was lower than in other seasons because of a low sea water temperature. After adding micro organisms, the quantity could meet G8 Guidelines requirements with the average of 1.64×10^6

and 1.43 × 10^6 CFU/100mL for two testing salinities. There was no significant difference between the quantity of inflow water to control tank and raw water. The quantity of discharged water from control tank after 5 days store was 3.67×10^5 CFU/100mL and 3.86×10^5 CFU/100mL, which was low than inflow water. The quantity of inflow water samples to treated tank with two salinities was 1.18×10^2 CFU/100mL and 42.6 CFU/100mL. There was no bacteria in testing cycle 2 and 3 of low salinity. After 5 days store, the quantity of discharged water from treated tank was 9.94 CFU/100mL and 6.8 CFU/100mL.No bacteria was existed in 21 samples during the 10 cycles.

表 3.7 陆基试验异养细菌测定结果（CFU/100mL）

Table 3.7 Quantity of heterotrophic bacteria for land-based testing

Quantity（CFU/100ml）					
High Salinity	Raw Water（RC-0）	Control Tank		Treated Tank	
		Inflow Water（R-0）	Discharged Water（C-5）	Inflow Water（T-0）	Discharged Water（T-5）
I	$1.13\times10^6\pm1.27\times10^5$	$1.23\times10^6\pm3.21\times10^5$	$3.07\times10^5\pm7.37\times10^4$	$1.20\times10^2\pm2.0\times10$	9.33 ± 16.2
II	$1.20\times10^6\pm1.73\times10^5$	$1.37\times10^6\pm3.79\times10^4$	$2.77\times10^5\pm1.56\times10^5$	$5.33\times10\pm9.24\times10$	6.67 ± 11.5
III	$3.03\times10^6\pm2.66\times10^6$	$1.60\times10^6\pm4.58\times10^5$	$3.83\times10^5\pm1.61\times10^5$	$1.67\times10^2\pm1.53\times10^2$	24.7 ± 47.2
IV	$1.30\times10^6\pm3.61\times10^5$	$1.10\times10^6\pm1.00\times10^5$	$5.17\times10^5\pm1.06\times10^5$	$1.12\times10^2\pm3.18\times10$	0
V	$1.53\times10^6\pm4.93\times10^5$	$1.26\times10^6\pm2.62\times10^5$	$3.50\times10^5\pm9.17\times10^4$	$1.37\times10^2\pm7.23\times10$	9.0 ± 15.6
AVG	1.64×10^6	1.31×10^6	3.67×10^5	1.18×10^2	9.94
Low Salinity					
I	$1.63\times10^6\pm6.66\times10^5$	$1.40\times10^6\pm2.00\times10^5$	$3.83\times10^5\pm7.64\times10^5$	$9.43\times10\pm4.86$	12.0 ± 10.6
II	$1.53\times10^6\pm1.53\times10^5$	$1.43\times10^6\pm2.52\times10^5$	$3.50\times10^5\pm1.50\times10^5$	0	0
III	$1.40\times10^6\pm2.65\times10^5$	$1.53\times10^6\pm2.52\times10^5$	$3.30\times10^5\pm1.05\times10^5$	0	0
IV	$1.37\times10^6\pm1.15\times10^5$	$1.23\times10^6\pm1.53\times10^5$	$4.27\times10^5\pm2.00\times10^5$	$7.13\times10\pm7.00\times10$	11.3 ± 19.6
V	$1.23\times10^6\pm5.77\times10^4$	$1.20\times10^6\pm1.00\times10^5$	$4.40\times10^5\pm7.94\times10^4$	$4.73\times10\pm4.10\times10$	10.7 ± 11.0
AVG	1.43×10^6	1.36×10^6	3.86×10^5	4.26×10	6.8

3.7 Human Pathogens

3.7.1 Vibrio spp.

Vibrio spp. was determined by TCBS plate selective medium which was incubated for 24 hours at 37 ℃. It was depended on the color characteristics of the *Vibrio* to decide whether it was needed to do further purification and 0/139 *Vibrio* Suomin sense experiments. No *Vibrio* spp. was exited in the inflow water to treated tank, as demonstrated in Table 3.8. There was no significant difference between raw water to treated tank and control tank, while the value was a little higher in high salinity than low salinity with an average of 2.59×10^3 CFU/100ml and 1.93×10^3 CFU/100ml. No *Vibrio* spp. was exited in 14 water samples, nor in the ballasting and deballasting process.

表 3.8 陆基试验弧菌测定结果（CFU/100mL）

Table 3.8 Quantity of Vibrio spp. for land-based testing

Quantity(CFU/100ml)					
High Salinity	Raw Water（RC-0）	Control Tank		Treated Tank	
		Inflow Water（R-0）	Discharged Water（C-5）	Inflow Water T-0	Discharged Water T-5
I	$2.10\times10^3\pm1.60\times10^3$	$3.27\times10^3\pm1.50\times10^3$	$1.33\times10^3\pm6.66\times10^2$	0	0
II	$3.30\times10^3\pm3.40\times10^3$	$3.47\times10^3\pm2.73\times10^3$	$1.53\times10^3\pm8.74\times10^2$	0	0
III	$2.67\times10^3\pm2.31\times10^3$	$2.77\times10^3\pm2.44\times10^3$	$1.93\times10^3\pm7.02\times10^2$	0	0
IV	$2.17\times10^3\pm1.68\times10^3$	$3.00\times10^3\pm2.65\times10^3$	$1.93\times10^3\pm7.23\times10^2$	0	0
V	$1.33\times10^3\pm1.15\times10^3$	$1.83\times10^3\pm7.64\times10^3$	$3.70\times10^3\pm1.45\times10^3$	0	0
AVG	2.31×10^3	2.87×10^3	2.08×10^3	0	0
Low Salinity					
I	$1.17\times10^3\pm1.26\times10^3$	$8.33\times10^2\pm7.64\times10^2$	$9.33\times10^4\pm1.01\times10^3$	0	0
II	$2.50\times10^3\pm1.06\times10^3$	$1.67\times10^3\pm7.02\times10^2$	$1.20\times10^3\pm1.08\times10^3$	0	0
III	$2.17\times10^3\pm2.02\times10^3$	$2.17\times10^3\pm1.89\times10^3$	$1.93\times10^3\pm7.02\times10^2$	0	0
IV	$2.83\times10^3\pm2.47\times10^3$	$3.50\times10^3\pm3.04\times10^3$	$1.80\times10^3\pm3.61\times10^2$	0	0

V	$1.50\times10^3\pm1.32\times10^3$	$1.00\times10^3\pm1.00\times10^3$	$1.73\times10^3\pm7.23\times10^2$	0	0
AVG	2.03×10^3	1.83×10^3	1.52×10^3		0

3.7.2 Escherichia coli

As demonstrated in Table 3.9, the quantity of *Escherichia col*i was 170 CFU/100mL and 105 CFU/100mL in raw water of high and low salinity, while that in inflow water and discharged water of control tank was below than 1×10^2 CFU/100mL. No *Escherichia col*i was exited in inflow water and discharged water of treated tank.

Table 3.9 Quantity of *Escherichia col*i for land-based testing

Quantity（CFU/100ml）					
High Salinity	Raw Water（RC-0）	Control Tank		Treated Tank	
		Inflow Water（R-0）	Discharged Water（C-5）	Inflow Water（T-0）	Discharged Water（T-5）
I	$2.73\times10^2\pm3.06\times10^1$	$2.77\times10^2\pm3.21\times10^1$	$1.30\times10^2\pm2.65\times10^1$	0	0
II	$2.20\times10^2\pm4.00\times10^1$	$2.60\times10^2\pm1.57\times10^2$	$1.20\times10^2\pm3.00\times10^1$	0	0
III	$1.03\times10^2\pm3.79\times10^1$	$1.27\times10^2\pm2.52\times10^1$	$1.47\times10^2\pm1.53\times10^1$	0	0
IV	$1.07\times10^2\pm6.11\times10^1$	$9.00\times10^1\pm3.00\times10^1$	$1.17\times10^2\pm4.51\times10^1$	0	0
V	$1.47\times10^2\pm1.53\times10^1$	$2.67\times10^2\pm8.74\times10^1$	$1.10\times10^2\pm3.00\times10^1$	0	0
AVG	1.70×10^2	2.04×10^2	1.25×10^2	0	0
Low Salinity					
I	$3.67\times10^1\pm4.04\times10^1$	$1.03\times10^2\pm4.93\times10^1$	$1.17\times10^2\pm3.51\times10^1$	0	0
II	$1.77\times10^2\pm2.52\times10^1$	$2.03\times10^2\pm4.04\times10^1$	$1.27\times10^2\pm3.21\times10^1$	0	0
III	$7.00\times10^1\pm6.56\times10^1$	$1.20\times10^2\pm2.00\times10^1$	$1.50\times10^2\pm5.57\times10^1$	0	0
IV	$9.67\times10^1\pm8.50\times10^1$	$1.10\times10^2\pm1.00\times10^1$	$1.73\times10^2\pm4.04\times10^1$	0	0
V	$1.43\times10^2\pm1.53\times10^1$	$9.00\times10^1\pm7.81\times10^1$	$1.53\times10^2\pm5.69\times10^1$	0	0
AVG	1.05×10^2	1.25×10^2	1.44×10^2	0	0

3.7.3 Intestinal Enterococci

The quantity of *Intestinal Enterococci* in the tested sea area is low in winter, therefore it was below 40 CFU/100ml in raw water and inflow water to control tank. No *Intestinal Enterococci* was existed in 20

samples, especially in all the samples of 3 cycles. No *Intestinal Enterococci* was existed in discharged water in 6 cycles for control tank. No *Intestinal Enterococci* was existed in all the inflow water and discharged water of treated tank.

Table 3.10 Quantity of *Intestinal Enterococci* for land-based testing

Quantity（CFU/100ml）					
		Control Tank		Treated Tank	
High Salinity	Raw Water（RC-0）	Inflow Water（R-0）	Discharged Water（C-5）	Inflow Water（T-0）	Discharged Water（T-5）
I	0	0	0	0	0
II	0	0	0	0	0
III	11.7±10.4	6.67±5.77	0	0	0
IV	16.7±15.3	10.0±10.0	7.67±4.04	0	0
V	10.3±4.51	7.67±4.04	17.7±13.7	0	0
AVG	7.7	4.87	5.07	0	0
Low Salinity					
I	10±10	3.33±5.77	0	0	0
II	0	0	0	0	0
III	23.3±20.8	13.3±11.5	0	0	0
IV	10±17.3	16.7±15.3	26.7±25.2	0	0
V	13.3±41.5	20±17.3	33.3±30.6	0	0
AVG	11.3	7.1	12.0	0	0

4. CONCLUSION & ASSESSMENT

Land-based testing of Elite Marine Equipment & Engineering Inc. was conducted in floating dock of COSCO (Dalian) Shipyard 16th~27th March, 2012. We compared the samples testing results with D-2 Standard together with G8 requirement and concluded as follow:

1. It was between the end of winter and the beginning of spring to do the testing. Temperature was around 1.5 ~ 3.1℃. The salinity fluctuation

among cycles was little. The difference among High Salinity Cycles (33.3~33.6 PSU) was just 0.4 PSU. Meanwhile the difference among Low Salinity Cycles (22.4~22.6 PSU) was just 0.3 PSU. Therefore, it was in accordance with G8 requirement.

2. The concentration of TSS in row water was almost the same as control tank inflow water. The average of the High Salinity Cycles was 1.78 mg/L, while the Low Salinity Cycles was 51.02mg/L. Therefore, these two groups' TSS concentration met G8 requirement. Moreover, the DOC concentration average of High Salinity Cycles and Low Salinity Cycles was 1.39 mg/L and 5.35 mg/L, this also met G8 requirement. Besides, the POC concentration average of High Salinity Cycles and Low Salinity Cycles was 1.17 mg/L and 5.17 mg/L. As a result, these main water quality indexes met G8 requirement.

3. Organism of $\geqslant$50μm size-range mainly were *Thalassiosira spp.* in diatom and added *Artenia salina* and *Brachionus.* There was no big difference in quantity and density of the two salinity raw water and control inflow water, high salinity in 1.67×10^5 ind./m^3 and 1.70×10^5 ind./m^3, and low salinity in 1.66×10^5 ind./m^3 and 1.64×10^5 ind./m^3. In deballasting water, the quantity of cultured *Artenia salina* and *Brachionus sp*. decreased largely. Therefore, it met G8 requirement. The living organism density (T-0) average of high and low salinity was 12.4 ind./m^3 and 13.20 ind./m^3. 5 days later, no living organism was detected

in high and low salinity in the second treatment seawater. It also met D-2 Standard.

4. The 10~50μm density average of organism in High Salinity Cycles row water and control tank inflow water was 1161.9cell/mL, while Low Salinity Cycles was 1136.5×10^5cell/mL. During deballasting 5 days later, the cell density of the control tank decreased 1 order of magnitudes which caused by decreasing of cultured algae. The average is 121cell/mL and 119.4cell/mL. The living organism density (T-0) average of high and low salinity was 6.9 cell/mL and 2.9 cell/mL. 5 days later, no living organism was detected in Low Salinity Cycles, while living organism in the third and the forth High Salinity Cycles was detected, but the average is 0.005cell/mL only. It also met D-2 Standard.

5. There are little heterotrophic bacteria and pathogenic bacteria in seawater, but it meets G8 requirement through adding extra heterotrophic bacteria. The average of bacteria in row water was 1.64×10^6CFU/100ml and 1.43×10^6 CFU/100mL separately in High Salinity Cycles and Low Salinity Cycles, while the inflow water of control tank was at the same level with the row water. 5 days later, the average in treated tank was 9.94CFU/100ml and 6.8CFU/100mL separately in High Salinity Cycles and Low Salinity Cycles after the second treatment. The quantity of Escherichia coli in row water was 170 CFU/100mL in High Salinity Cycles and 105 CFU/100mL in Low Salinity Cycles. Furthermore, there

was no Escherichia coli detected both in inflow water and discharged water. The quantity of enterococcus was very low, and fluctuated between 0~40 CFU/100ml both in row water and inflow water of control tank of High and Low Salinity Cycles. There were 20 samples failed to culturing bacterial colony, and no enterococcus was detected in 3 cycles' samples. Moreover, there was no enterococcus detected in both inflow water of treated tank and discharged water, also. The density of Vibrions in inflow water was 2.59×10^3 CFU/100ml and 1.93×10^3 CFU/100ml separately. There were 14 samples failed to culturing bacterial colony, and T-0 treated tank was the same results. Therefore, these four microorganisms were completely in conformity with D-2 standard for treated water.